<Division of Biochemistry>Chemistry of Molecular Biocatalysts

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– Chemistry of Molecular Biocatalysts –

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Prof
YAMAGUCHI, Shinjiro
(D Agr)

Assist Prof
WATANABE, Bunta
(D Agr)

Assist Prof
MASHIGUCHI, Kiyoshi
(D Agr)

PD
TAKEMOTO, Kodai
(D Sc)

Students

ISHIDA, Toshiaki (D1)
ISHIWARI, Kenji (M2)
HIRANO, Rena (M2)
KOBAYASHI, Takahiro (M1)
TAKEMOTO, Kiyoko (M1)
HASE, Kanako (M1)

Scope of Research

Plant hormones are a group of small molecules that are synthesized by plants and control their growth, development and environmental responses. This laboratory aims at elucidating how plant hormones are made and act in plants. Towards this goal, we combine chemical (organic chemistry, biochemistry, and analytical chemistry) and biological (molecular genetics, physiology, molecular biology, genomics) approaches. We are also looking for new hormone-like compounds by using mutant plants that show morphological phenotypes.

KEYWORDS

Plant Hormone
Strigolactone
Biosynthesis
Cytochrome P450
Receptor

Selected Publications

Yasui, R.; Seto, Y.; Ito, S.; Kawada, K.; Itto-Nakama, K.; Mashiguchi, K.; Yamaguchi, S., Chemical Screening of Novel Strigolactone Agonists that Specifically Interact with DWARF14 Protein, *Bioorg. Med. Chem. Lett.*, **29,** 938-942 (2019).

Seto, Y.; Yasui, R.; Kameoka, H.; Tamiru, M.; Cao, M.; Terauchi, R.; Sakurada, A.; Hirano, R.; Kisugi, T.; Hanada, A.; Umehara, M.; Seo, E.; Akiyama, K.; Burke, J.; Takeda-Kamiya, N.; Li, W.; Hirano, Y.; Hakoshima, T.; Mashiguchi, K.; Noel, J. P.; Kyozuka, J.; Yamaguchi, S., Strigolactone Perception and Deactivation by a Hydrolase Receptor DWARF14, *Nat. Commun.*, **10,** [191-1]-[191-10] (2019).

Burger, M.; Mashiguchi, K.; Lee, H. J.; Nakano, M.; Takemoto, K.; Seto, Y.; Yamaguchi, S.; Chory, J., Structural Basis of Karrikin and Non-natural Strigolactone Perception in *Physcomitrella patens*, *Cell Rep.*, **26,** 855-865 (2019).

Mashiguchi, K.; Hisano, H.; Takeda-Kamiya, N.; Takebayashi, Y.; Ariizumi, T.; Gao, Y.; Ezura, H.; Sato, K.; Zhao, Y.; Hayashi, K.; Kasahara, H., *Agrobacterium tumefaciens* Enhances Biosynthesis of Two Distinct Auxins in the Formation of Crown Galls, *Plant Cell Physiol.*, **60,** 29-37 (2019).

The Mechanism for Strigolactone Perception and Deactivation by a DWARF14 Receptor

Strigolactones (SLs) have been identified as root-derived signals for parasitic and symbiotic interactions in the rhizosphere. In addition to these functions as allelochemicals, SLs are now known as endogenous plant hormones that regulate many aspects of plant growth such as outgrowth of axillary shoots, root development and leaf senescence.

Recently, the perception mechanism for SLs has been a subject of debate because their receptor, DWARF14 (D14), is an α/β-hydrolase that can cleave SLs. In the study of Seto *et al.* (2019), we first conducted time-course analyses of SL binding and hydrolysis by Arabidopsis D14 (AtD14) by using differential scanning fluorimetry (DSF) experiments and hydrolysis assays (Figure 1). In these analyses, we found that the level of uncleaved GR24, a synthetic SL analog, strongly correlates with the induction of the melting temperature (Tm) shift of AtD14, suggesting that SLs trigger the active state of D14 before their hydrolysis.

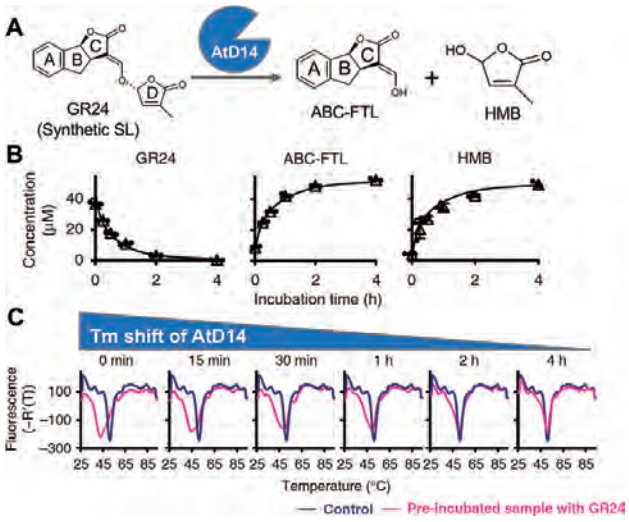

Figure 1. The time-course analyses of SL binding and hydrolysis by AtD14. (A) Hydrolysis reaction of SL (GR24) by AtD14. (B) Quantification of the hydrolysis reaction of GR24 (40 μM) by AtD14. (C) The Tm shift of AtD14 incubated with GR24 (40 μM) in DSF analysis. The maximum Tm shift was observed at initial incubation (0 min).

We next analyzed the catalytic triad mutants of AtD14. Among them, interestingly, the AtD14^{D218A} mutant completely complemented the hyper-branching phenotype of the *atd14* mutant though it lacks the hydrolase activity (Figure 2). Moreover, we found that the AtD14^{D218A} mutant could interact with SMXL7, a negative regulator of SL signaling, in an SL-dependent manner in yeast two hybrid experiments (Figure 2). These findings suggest that the hydrolase reaction catalyzed by D14 is not necessary for the signal transducing role.

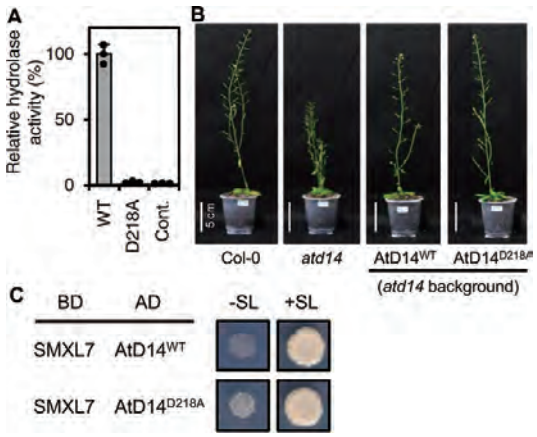

Figure 2. Functional analysis of the AtD14^{D218A} mutant. (A) The SL hydrolysis activities of maltose binding protein (MBP)-tagged wild-type of AtD14 protein (AtD14^{WT}) and the AtD14^{D218A} mutant. MBP was used in the control reaction. (B) The branching phenotype of Arabidopsis transgenic plants expressing AtD14^{WT} and AtD14^{D218A}. (C) The SL-dependent interaction of SMXL7 with AtD14^{WT} and AtD14^{D218A} in yeast.

Moreover, we showed that overexpression of the AtD14^{R183H} mutant, which could not interact with SMXL7 in the presence of SLs, but could hydrolyze SLs like AtD14^{WT}, resulted in an increased shoot branching phenotype (data not shown). These results support the idea that the hydrolase reaction catalyzed by D14 would be a deactivating step of SLs after transducing the signal.

From these lines of evidence, we conclude that the intact SL molecules trigger the D14 active signaling state, and that D14 deactivates bioactive SLs by hydrolysis after signal transmission. Together, our results revealed that D14 is a dual-functional receptor, responsible for both the perception and deactivation of bioactive SLs (Figure 3).

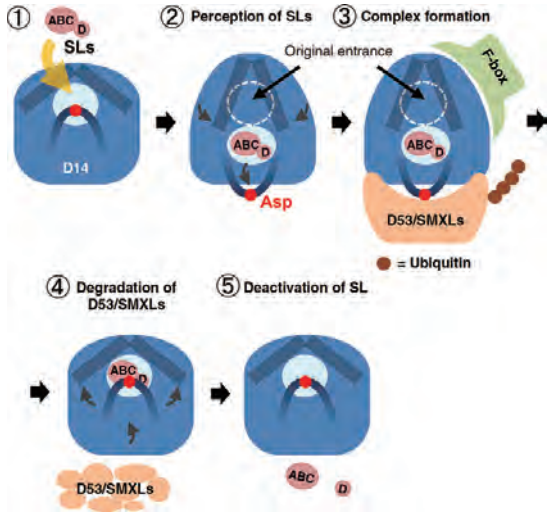

Figure 3. A proposed model of D14 in SL signaling. Bioactive SLs induce the conformational changes of D14, which triggers complex formation with the signaling partners. After the degradation of negative regulators of SL signaling (D53/SMXLs) and transmission of the SL signal, D14 reconstructs the catalytic triad to inactivate the bioactive SL.